Improved detection of unique transcripts from FFPE-derived RNA using a novel, rapid whole transcriptome sequencing workflow

Genomics

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"FFPE samples are an invaluable resource for oncology researchers, providing access to a vast library of diseased tissue samples paired with relevant donor information. Despite their broad utility, FFPE-derived RNA samples often vary wildly in performance, with fixation process, block age and storage, and extraction methodology having large impacts on resulting template quality. As a result, robust and reproducible RNA sequencing with FFPE-derived RNA remains a challenge.

To address this need, we developed a novel, streamlined whole-transcriptome library preparation workflow specifically tailored for processing degraded samples. To minimize off-target effects, traditional RNA depletion employs a lengthy DNA probe hybridization and the subsequent addition of RNase H at an elevated temperature. This complicates the workflow and makes it difficult to automate. We hypothesized that probe design, incubation time, and probe concentration impact off-target depletion. To simplify the protocol while minimizing off-target effects, we built algorithms for optimal probe design and developed our workflow to support efficient depletion from degraded samples without the need for a separate probe hybridization step. Additionally, we dramatically improved yields and performance with degraded samples by incorporating a decrosslinking step, engineered enzymes, and uniquely optimized reaction formulations. Reducing incubation times and eliminating cleanup steps streamlined the downstream library preparation workflow.

With RNA extracted from five FFPE blocks, we compared our solution to commercial products using 100 ng inputs. Libraries generated using our solution resulted in improved library yields, unique transcript identification, strand specificity, and rRNA depletion efficiency on a per-block basis. Technical replicates showed excellent transcript abundance correlation. Additionally, we observed less than 1% residual rRNA and globin mRNA with no measurable off-target effects using a blood-derived, high-quality sample with input amounts as low as 10 ng. Our novel, simplified workflow yields robust and reproducible performance with FFPE samples while enabling library construction within five hours."